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Mosaic vs. Nonmosaic Trisomy 9: Report of a Liveborn Infant Evaluated by Fluorescence In Situ Hybridization and Review of the Literature

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We report on a newborn infant with multiple congenital anomalies and apparent nonmosaic trisomy 9 in the blood (by conventional cytogenetic studies) who died shortly after birth. Clinical observations at birth and autopsy are compared with phenotypes of mosaic and nonmosaic trisomy 9 cases reported previously. Unlike the initial cytogenetic analysis, fluorescence in situ hybridization (FISH) studies of metaphase and interphase blood cells and skin fibroblasts detected the presence of euploid and trisomy 9 cells. These results suggest that earlier reports of trisomy 9, which relied on conventional chromosome analysis of a few metaphase cells and/or only one tissue type, may not have excluded mosaicism, and that trisomy 9 may be viable only in the mosaic state. © 1996 Wiley-Liss, Inc.

KEY WORDS: trisomy 9, mosaicism, FISH, congenital defects

INTRODUCTION

Trisomy 9 is a relatively common finding in spontaneous abortions [Jacobs, 1977], is rare in liveborn infants, and in many cases appears to represent mosaics. Clinical observations at birth and autopsy of a male newborn infant are compared with reported cases of mosaic and nonmosaic trisomy 9 liveborn infants, while results from cytogenetic and fluorescence in situ hybridization (FISH) studies of blood leukocytes and skin fibroblasts are evaluated in an effort to identify how to effectively detect mosaicism.

MATERIALS AND METHODS

Clinical Presentation and Autopsy Findings

This 37-week, 1,970 g, male newborn infant was delivered by emergent cesarean section for fetal distress with polyhydramnios, intrauterine growth retardation, and breech presentation to a 30-year-old gravida 2, para 2 white woman. The infant had poor respiratory effort and bradycardia with Apgar scores 2, 3, and 3 at 1, 5, and 10 min, respectively. A large left diaphragmatic hernia and multiple minor anomalies suggested a nonviable chromosomal abnormality, and the infant died shortly after bag and mask ventilation was discontinued. On physical examination (Fig. 1), weight and length were <10th centile, and occipitofrontal circumference (OFC) was at the 70th centile. Examination also showed wide sagittal suture (3 cm), frontal bossing, small mouth, deep-set eyes, bulbous nose (Fig. 1a), depressed left globe, bilateral microphthalmia, cataracts, corneal clouding, malformed, apparently low-set ears (Fig. 1b) with no left external auditory canal, large cleft palate, and hypoplastic mandible and maxilla. Also noted were micropenis, bilateral cryptorchidism, small scrotum, and hypoplastic anus (Fig. 1c). Figure 1d–f shows flexion contractures of the fingers, index and fifth fingers overlapping other digits, hypoplastic nails, and rocker-bottom feet in a valgus position. A short (10-cm) umbilical cord with single umbilical artery was present. Radiographically, there were prominent cervical vertebral malformations and malformed sacrum. At autopsy, a hypoplastic gallbladder, membranous atrial septal defect, patent urachus with cyst, a dysplastic left kidney with retention cyst, and hypertrophied right kidney were found. The brain was grossly normal.

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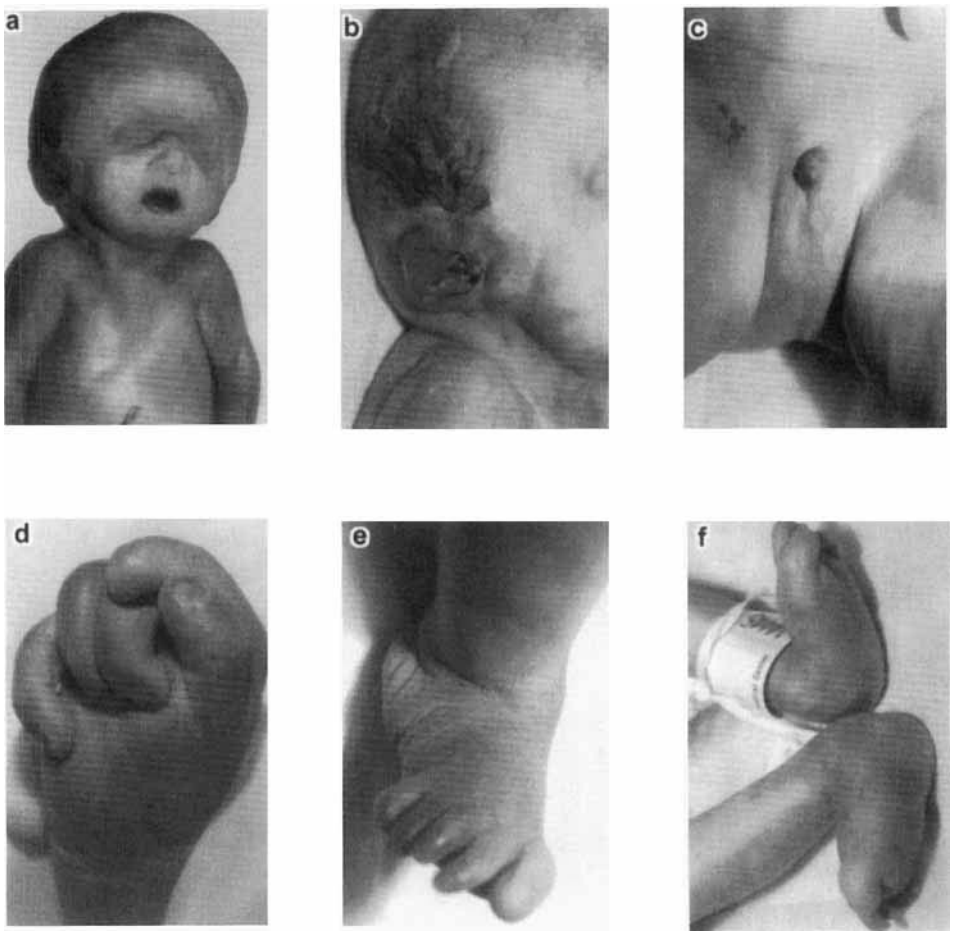

Fig. 1. **a–f:** Infant before and during autopsy examination, showing clinical manifestations described in the text.

Sixteen of the most commonly documented clinical signs were evaluated as present (+), absent (−), or unknown(?), in 44 cases of trisomy 9, from 37 publications and from our present case (Table I). Fisher's exact test was used for statistical analysis of the frequencies of (+) and (−) determinations in 28 mosaic cases and in 16 cases considered to be nonmosaics.

Cytogenetic Studies

Blood and fibroblast cultures from a skin biopsy were prepared for GTG-banded chromosome analysis [Seabright, 1971]. Sixty metaphases from each cell type were studied, to exclude a second cell line with a frequency of ≥5% at a 95% confidence level [Hook, 1977].

FISH Studies

FISH analyses were performed using a biotin-labeled chromosome 9-specific classical satellite DNA probe having sequence homology to locus D9Z1 (Cat. #P5016, Oncor, Inc., Gaithersburg, MD). The in situ hybridization experiments were performed on preparations of peripheral lymphocytes and cultured skin fibroblasts from the infant, and followed the manufacturer's FISH protocol. Normal lymphocytes and skin fibroblasts served as control cells for the FISH technique.

RESULTS

Cytogenetic results showed that all 60 blood lymphocytes were trisomic for chromosome 9 (Fig. 2). Subsequent study of 60 fibroblast cells demonstrated two karyotypically normal cells which suggested the possibility of low-grade mosaicism. FISH results on metaphases from 60 lymphocytes and 60 fibroblasts are presented in Table II, and agree with GTG-banding studies, demonstrating three signals for chromosome 9 in all 60 lymphocytes and in 58 of 60 (96.7%) fibroblasts. However, FISH studies of over 600 interphase cells showed that 108 of 683 (16%) lymphocytes and 56 of 625 (9%) fibroblasts examined had only two signals, indicating a normal 46,XY cell line in addition to the predominant trisomy 9 cell line. The extent of mosaicism shown by FISH of interphase cells is greater

TABLE I. Summary and *P* Level Evaluations of Phenotypic Abnormalities Reported for Mosaic and Nonmosaic Trisomy 9 Liveborns, and Comparison to Present Case

	Phenotypic abnormalities[a]															
Reference	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mosaic																
Haslam et al., 1973	+	+	?	−	+	−	−	+	−	+	−	+	+	+	?	?
Bowen et al., 1974	−	+	?	−	−	−	−	+	−	+	+	+	+	+	−	+
Sutherland et al., 1976	−	?	+	+	+	+	−	?	−	+	−	+	+	+	+	?
	+	?	+	?	+	?	?	+	?	+	?	?	+	+	?	+
Lewandowski and Yunis, 1977	+	?	?	+	+	?	−	+	−	+	−	+	+	+	−	−
Tropp and Currie, 1977	−	−	?	+	+	?	?	+	?	−	?	+	−	+	?	?
	−	−	?	−	+	?	?	−	?	−	?	−	−	−	?	?
Qazi et al., 1977	−	+	?	−	+	+	−	−	−	+	?	+	+	+	+	−
Akatsuka et al., 1979	−	+	−	+	+	−	−	−	−	+	−	−	+	−	−	+
Katayama et al., 1980	+	+	+	+	+	?	+	+	−	+	?	+	+	?	+	−
Frydman et al., 1981	?	+	?	?	?	?	?	?	?	?	?	+	+	?	?	+
Annerén and Sedin, 1981	−	+	−	−	+	−	−	−	−	+	−	−	+	+	−	−
Ginsberg et al., 1982	?	+	?	+	+	?	+	?	?	+	?	+	?	+	?	+
Sánchez et al., 1982	−	?	+	+	+	?	+	+	?	+	?	+	?	+	+	−
Wilson and Barr, 1983	−	?	?	−	+	−	−	?	−	+	+	−	+	+	+	?
Kaminker et al., 1985	−	+	−	−	+	−	−	+	+	−	?	−	+	?	+	?
Herranz et al., 1987	+	?	?	−	−	−	−	+	−	−	?	−	+	−	−	−
Levy et al., 1989	−	+	+	+	+	?	?	?	?	−	+	+	+	+	+	+
Ginsberg et al., 1989	?	+	?	+	+	?	?	?	+	+	?	+	+	+	+	?
Stoll et al., 1993	−	?	−	−	+	−	−	?	−	+	?	?	+	?	−	?
	−	+	−	−	+	−	−	?	−	+	?	+	+	+	−	?
Willatt et al., 1992	−	?	?	?	+	−	−	?	+	+	?	+	+	?	−	?
De Michelena et al., 1992[b]	−	+	+	+	−	−	−	?	−	+	+	+	+	+	−	−
Sherer et al., 1992	−	?	?	?	+	−	−	?	−	+	+	+	?	+	?	?
Arnold et al., 1995	−	−	+	−	+	?	−	+	−	−	−	+	+	+	+	−
	−	+	−	+	+	?	−	+	+	+	−	+	+	−	+	−
Wooldridge and Zunich, 1995	−	+	−	−	+	?	−	−	−	+	−	−	+	−	−	−
Nonmosaic																
Feingold and Atkins, 1973	+	?	?	−	+	+	−	?	−	+	−	+	+	+	+	+
Kurnick et al., 1974	−	−	?	−	+	+	−	+	+	+	−	+	+	+	−	−
Seabright et al., 1976	?	?	+	?	+	?	?	?	?	+	+	?	+	+	?	?
Annerén and Sedin, 1981	−	+	+	−	+	−	−	−	−	+	+	+	+	+	+	−
Mantagos et al., 1981	−	+	?	?	+	−	?	?	?	+	+	+	+	+	+	+
	−	+	+	?	+	−	?	?	?	+	+	+	+	+	+	+
Carpenter and Tomkins, 1982	−	−	+	−	+	?	−	?	+	+	+	+	+	+	−	+
Pfeiffer and Müller, 1984	−	+	+	+	+	−	?	+	+	−	−	+	+	+	+	+
Delicado et al., 1985	−	?	?	−	+	−	−	?	−	+	+	+	+	+	−	−
	?	?	?	?	−	?	?	?	?	?	?	−	+	?	?	+
Williams et al., 1985	+	+	+	?	+	?	?	?	?	?	?	+	?	?	?	+
Marino et al., 1989	−	?	?	+	+	+	−	?	+	+	?	+	+	+	+	+
Benacerraf et al., 1992	−	+	+	?	+	?	?	?	?	+	+	+	+	+	+	?
Roberts et al., 1993	−	+	+	?	+	−	−	?	−	+	+	−	+	+	+	+
Golden and Schoene, 1993	+	+	?	?	+	?	?	?	?	+	+	?	+	+	?	−
	+	+	+	?	+	?	?	?	?	+	+	?	+	+	+	+
Present case	−	+	+	+	+	−	?	−	+	+	+	+	+	+	+	+
Mosaic																
+	5	16	8	12	24	2	3	11	5	21	6	19	23	18	11	7
−	20	3	7	12	3	13	18	6	16	6	8	7	2	5	10	10
?	3	9	13	4	1	13	7	11	7	1	14	2	3	5	7	11
Nonmosaic																
+	4	9	9	2	15	3	0	2	4	13	10	11	15	14	9	10
−	10	2	0	5	1	6	7	1	4	1	3	2	0	0	3	4
?	2	5	7	9	0	7	9	13	8	2	3	3	1	2	4	2
P level values	.70	1.0	.02	.41	1.0	.33	.55	1.0	.21	.39	.12	.69	.52	.13	.28	.15

[a] Phenotypic abnormalities: 1, microcephaly; 2, cleft/arched palate; 3, wide fontanelles; 4, deep-set eyes; 5, low-set ears; 6, low hairline; 7, small mouth; 8, abnormal palmar crease; 9, facial asymmetry; 10, micrognathia; 11, congenital renal disease; 12, bulbous nose; 13, skeletal anomalies; 14, congenital heart disease; 15, microphthalmia; 16, underdeveloped genitalia.
[b] This case may represent a misdiagnosed trisomy 8.

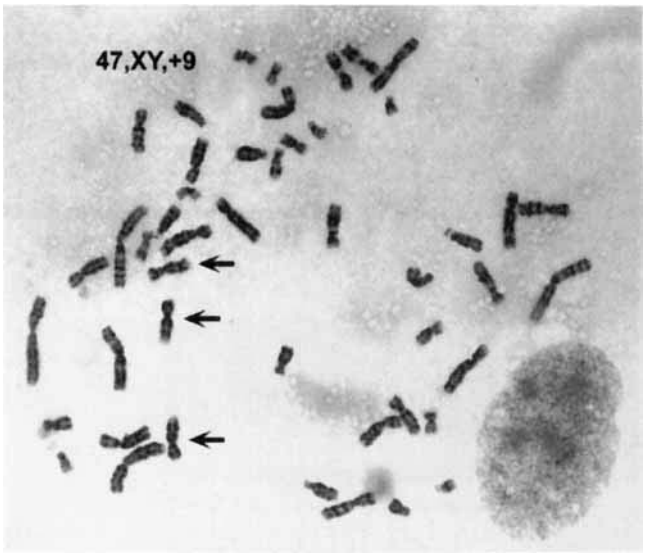

Fig. 2. Photomicrograph of metaphase cell, showing trisomy 9 by conventional cytogenetic methods (GTG-banding). Arrows point to three chromosomes 9.

than the 3.3% indicated by either conventional cytogenetic or FISH analysis of 60 fibroblasts metaphases, and may have been missed altogether if only conventional cytogenetic studies of blood lymphocytes had been provided. In control studies for the FISH protocol, only 5 of 305 normal interphase lymphocytes (1.6%) and 12 of 312 normal interphase fibroblasts (3.8%) had different hybridization signals than the expected diploid complement. Therefore, these FISH studies indicate a diagnosis of mosaic trisomy 9: 46,XY/47,XY,+9. Photomicrographs of FISH results are presented in Figure 3.

In Table I, results from Fisher's exact tests show there were no statistically significant differences between the mosaic and nonmosaic categories for 15 of 16 clinical findings. One anomaly (wide fontanelles) appeared to be significantly different ($P = .02$), but this result is well within expectations for type I statistical error, and any significant difference between mosaic and nonmosaic cases disappeared when the 16 probabilities were summed using the method of Fisher ($\chi^2 = 35.3$, 32 *df*). Thus, it does not appear possible to distinguish a mosaic from a nonmosaic trisomy 9 case, based on clinical findings alone.

DISCUSSION

Our study suggests that karyotypic mosaicism of trisomy 9 may not have been resolved by earlier studies which used routine cytogenetic testing limited to lymphocytes. Low-grade and/or tissue-specific mosaicism (such as found in our case) may be detectable only when the analysis is extended to include: 1) more metaphases, 2) metaphase cells from a second tissue (i.e., skin fibroblasts), and/or 3) FISH studies conducted on a much larger number of interphase cells from both tissue types.

Lack of significant differences in clinical phenotypes of mosaic vs. nonmosaic trisomy 9 also supports the idea of probable cryptic mosaicism in reported cases. Since mosaicism involving a normal cell line tends to have a less severe phenotype than nonmosaic cases, a greater difference in the clinical profile between mosaic and nonmosaic trisomy 9 was expected. Therefore, our analysis of clinical phenotypes reported in the literature supports the observation that chromosome 9 imbalances found in viable infants generally are partial trisomies, mosaics, or a combination of these [Schinzel, 1984], and that nonmosaic complete trisomy of chromosome 9 is inviable and rarely, if ever, found in liveborn infants.

The incidence of karyotypic mosaicism may be much higher than previously recognized, principally because earlier studies were limited by the available materials and techniques. The present case shows that FISH is more sensitive in detecting cryptic mosaicism, and that it should be applied to accurately characterize important genetic imbalances such as trisomy 9, in order to generate meaningful clinical correlations.

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TABLE II. FISH Results Demonstrating Trisomy 9 Mosaicism

Tissue type	Number of cells examined	Number of signals observed			
		≤1	2	3	≥4
Lymphocytes:					
Metaphases (%)	60	0 (0%)	0 (0%)	60 (100%)	0 (0%)
Interphase (%)	683	18 (2%)	108 (16%)	550 (81%)	7 (1%)
Control (%)[a]	305	5 (2%)	300 (98%)	0 (0%)	0 (0%)
Fibroblasts:					
Metaphases (%)	60	0 (0%)	2 (3%)	58 (97%)	0 (0%)
Interphases (%)	625	31 (5%)	56 (9%)	508 (81%)	30 (5%)
Control (%)[a]	312	12 (4%)	300 (96%)	0 (0%)	0 (0%)

[a]Interphase cells.

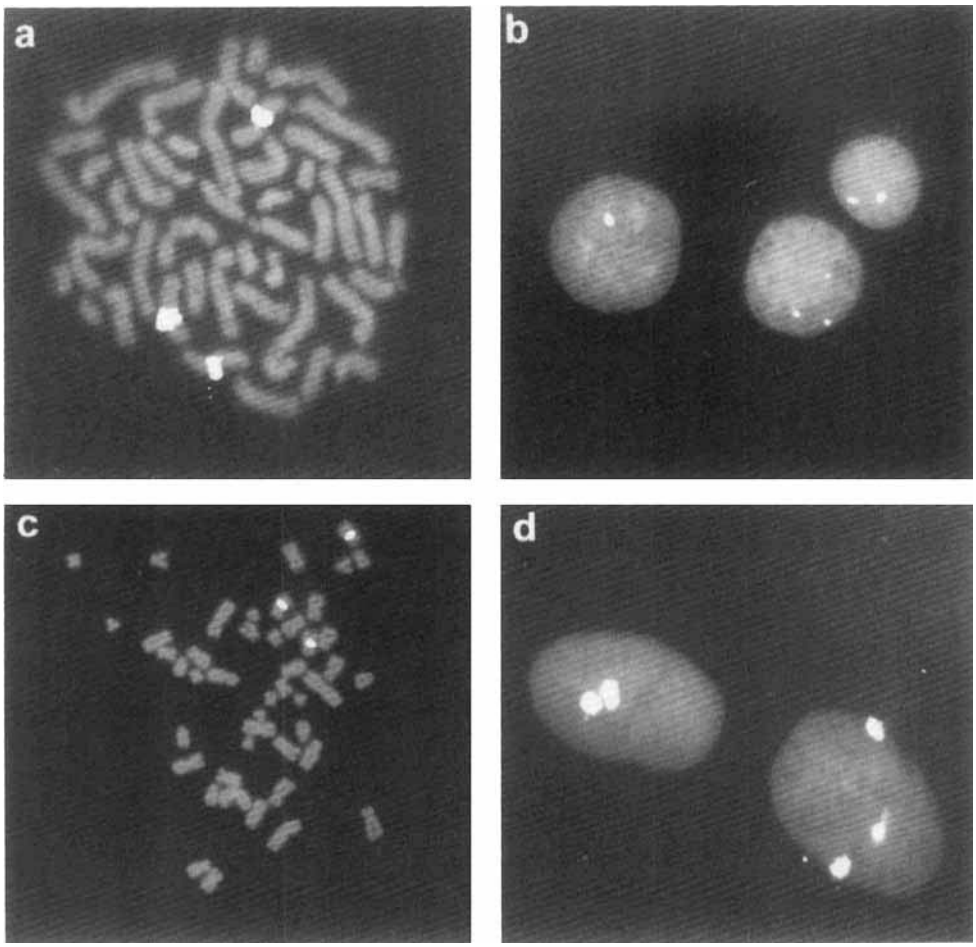

Fig. 3. Photomicrographs of FISH results, showing three and two signals in metaphase (**a**) and interphases (**b**) of blood lymphocytes, respectively; and in metaphase (**c**) and interphases (**d**) of skin fibroblasts, respectively, when chromosome 9-specific classical satellite DNA (locus D9Z1) probe was used.

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